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SHORT COMMUNICATIONS

TEMPERATURE DEPENDENT EMBRYOGENESIS IN *AESHNA TUBERCULIFERA* WALKER AND *PLATHEMIS LYDIA* (DRURY) UNDER FIELD AND LABORATORY CONDITIONS (ANISOPTERA: AESHNIDAE, LIBELLULIDAE)

T. HALVERSON
Department of Zoology, University of Maryland, College Park,
Maryland 20742, United States

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Hatching success and rate of embryonic development were measured at 7 constant temperatures in the laboratory and in 5 natural ponds in western Virginia, USA. Hatching success in the lab was reduced at low temperatures for *P. lydia* and at high temperatures for *A. tuberculifera*. These results are consistent with the phenologies and geographical distributions of these spp. Hyperbolic functions accurately described the affects of temperature on the logarithmic rate of embryogenesis for both spp. in the laboratory but temperature appeared to have little influence on either hatching success or embryogenic rate in the field. Thus other environmental factors must have accounted for the variation which existed among ponds in both hatching success and rate of embryonic development. Circumstantial evidence suggests that oxygen level may have been the important limiting factor in the ponds investigated in this study.

INTRODUCTION

The effects of temperature on developmental rates have been discussed for a large number of ectothermic organisms (HOWE, 1967; ELLIOTT & HUMPESCH, 1980; KANKAALA & WULFF, 1981; MacLAREN, 1965, 1966; TAYLOR , 1981; and others). Laboratory experiments have almost universally demonstrated a positive relationship between temperature and the rate of development over most of the natural temperature range of a species. It is not surprising, therefore, that differences in developmental rates which are observed among natural populations may be attributable to differences in the temperature regimes which the populations experience (TAYLOR, 1981).

In another paper (Halverson, in prep.) I report differences among a set of five neighboring ponds in the rates of embryonic development, hatching success, and larval growth and survival of *Aeshna tuberculifera* and *Plathemis lydia*. The field experiments which led to these findings involved monitoring sets of eggs reared in enclosures in each of the ponds. Because individual cohorts of eggs were represented equally in each pond, the observed variation was not the result of genetic differences, but rather, represented environmentally induced variation in these life-history parameters. In the current paper, I examine the extent to which temperature differences among the ponds may have been responsible for the observed differences in embryonic development rate and hatching success. I first discuss the results of controlled temperature experiments conducted in the laboratory, and then relate these findings to the results of the field experiments.

METHODS

Eggs of both *A. tuberculifera* and *P. lydia* were kept in the laboratory under seven temperature conditions in 20 dram, polystyrene vials filled with aerated tap water. *A. tuberculifera*, eggs were collected in March of 1980 from reed stems (*Juncus effusus* L.) at a series of small ponds in the Shenandoah Mountains, Rockingham Co., Virginia. *A. tuberculifera* is the only aeshnid species in this area which commonly deposits its eggs in *Juncus* stems above the water surface (HALVERSON, 1983). The identification of these eggs was further verified by rearing a number of the collected eggs through the 10th instar in field enclosures (Halverson, in review). Field collected *A. tuberculifera* eggs were assigned randomly, 5 eggs per vial, to each of 21 vials, providing three replicate vials (15 eggs) at each of the seven temperature treatments. *P. lydia* eggs were collected in late May 1980 from four recently mated females at the same mountain ponds. Seven groups of ten eggs from each of the four females were placed in their own vials thus providing four replicate vials, one for each female, (40 eggs) at each of the seven temperature treatments. All incubators were kept at a 12 hour light — 12 hour dark cycle for the duration of the experiment. The temperature treatments for the laboratory experiments are reported as the mean of daily temperature measurements taken from the first day of the experiment to the day on which the median egg hatched in each replicate vial.

Eggs from the same collections as those used in the laboratory were also maintained in field enclosures in each of five study ponds at the Shenandoah Mountain study site. These field enclosures were also constructed from 20 dram, polystyrene vials but had four 20 mm diameter holes drilled in the walls. Strips of Nitex screening (355 micron mesh) were glued in place over these holes with a solution of polystyrene chips dissolved in chloroform. Zooplanktors rapidly colonized these enclosures demonstrating the extensive exchange of water and microbiota between the pond and the enclosures.

Enclosures were anchored to metal stakes placed at equal intervals around the perimeter of the ponds at 50 to 100 cm centripitad to the shoreline. The low specific gravity of the polyethylene caps insured that the enclosures floated in an upright position just below the pond surface. Six stations were used at each pond with two enclosures per species per station. Three eggs were placed in each enclosure for *P. lydia* while each *A. tuberculifera* enclosure contained two eggs. All enclosures were monitored weekly at which time eggs were checked for hatching and the water temperature at each station was recorded. Maximum and minimum temperatures were also recorded weekly from Taylor max-min thermometers located at one of the stations in each pond.

Temperature differences among stations within a pond were tested with two-way analyses of variance using date as a blocking factor. For those ponds in which there were no significant temperature differences among stations, field temperatures were estimated for each species by taking the mean of the weekly maximum and minimum temperatures from the start of the experiment, up to and including the week in which the majority of eggs hatched. For ponds in which there were significant temperature differences among stations, these estimated field temperatures were adjusted by the average temperature difference between the station which housed the max-min thermometer and the stations at which eggs successfully hatched.

RESULTS

LABORATORY EXPERIMENTS

Rates of embryogenesis (Re) in the laboratory were determined as 1/median days to hatching for each replicate vial. Natural log transformations of this variable were necessary to reduce heterogeneity of variances among the temperature treatments. Hyperbolic functions of ln (Re) and temperature were found to explain more of the variation in embryogenic rate in the laboratory than linear, power, squared, or exponential functions for both species (Fig. 1, *A. tuberculifera:* $N = 21$, $r^2 = 0.970$; *P. lydia:* $N = 24$, $r^2 = 0.974$). Furthermore,

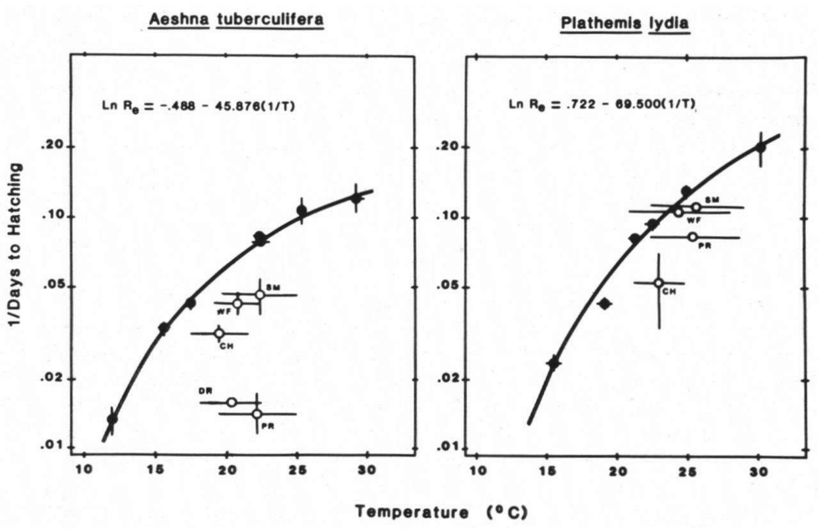

Fig. 1. Rate of embryogenesis as a function of temperature for *Aeshna tuberculifera* and *Plathemis lydia*. Solid symbols represent the mean Re (± 2 SE) from laboratory experiments in which temperature was controlled. Open circles represent values from the study ponds and are labelled accordingly. No *P. lydia* eggs hatched at DR pond so embryogenic rate could not be calculated. Pond temperatures are presented as the mean (± 1 SE) of the maximum and minimum temperatures recorded weekly during the period of embryogenesis. Only values from the laboratory experiments were used in calculating the regression equations.

the hyperbolic function was the only one of these standard functions which yielded a significant fit to the data in regression analyses of variance (*A. tuberculifera:* F-ratio for regression = 1278.58, $p<0.001$, F-ratio for lack of fit = 0.99, $p>0.25$; *P. lydia:* F-ratio for regression = 1496.05, $p<0.001$, F-ratio for lack of fit = 1.15, $p>0.25$).

Hatching success also showed a clear temperature dependence in the laboratory (Fig. 2). Fewer *A. tuberculifera* eggs hatched at temperatures above 25° C, while *P. lydia* eggs showed a drop in hatching success at temperatures below 18° C. Chi-square contingency table analyses in which temperatures were combined to form three temperature categories verified the significance of these differences for both species *A. tuberculifera:* $\chi^2 = 16.08$, df = 2, $p<0.01$; *P. lydia:* $\chi^2 = 104.82$, df = 2, $p<0.001$).

FIELD EXPERIMENTS

Temperatures did not differ consistently among the six stations within SM, DR, WF, or PR ponds, but a significant difference was found among the stations at CH pond (Tab. I). The max-min thermometer at CH pond was positioned at the coldest station, hence it was necessary to adjust the estimated field temperatures to correct for this bias. Since *A. tuberculifera* and *P. lydia* had different spatial distributions of egg hatching in CH pond, the adjustment was carried out separately for each species, and consisted of the addition of 1.86° C for *A. tuberculifera* and 1.18° C for *P. lydia.*

Rates of embryogenesis in the field were generally lower than in the laboratory at comparable temperatures for both species (Fig. 1). Only the values for *P. lydia*

Table I

Mean temperatures at the six stations within each pond averaged over the period from 27 May to 31 August — (Values within SM, DR, WF, and PR were not different from one another at a 0.05 significance level using SNK multiple range tests. Values which were significantly different within CH are indicated by different letters. Station 1 was the position of the max-min thermometer at each pond. Stations at which *A. tuberculifera* and *P. lydia* successfully hatched are indicated by the symbols "+" and "*" respectively)

Station No.	Pond SM	DR	CH	WF	PR
1	+* 24.33	20.36	* 20.61 a	+* 21.37	21.40
2	+* 24.67	20.00	+* 21.67 abc	+* 21.47	20.87
3	+* 25.75	20.41	* 23.08 cd	+* 21.67	21.30
4	+* 25.16	+ 20.14	+ 23.27 d	+* 21.53	* 20.63
5	+* 24.67	20.96	22.43 bcd	+* 21.37	21.70
6	+* 25.36	21.19	20.82 ab	+* 21.27	+ 21.50

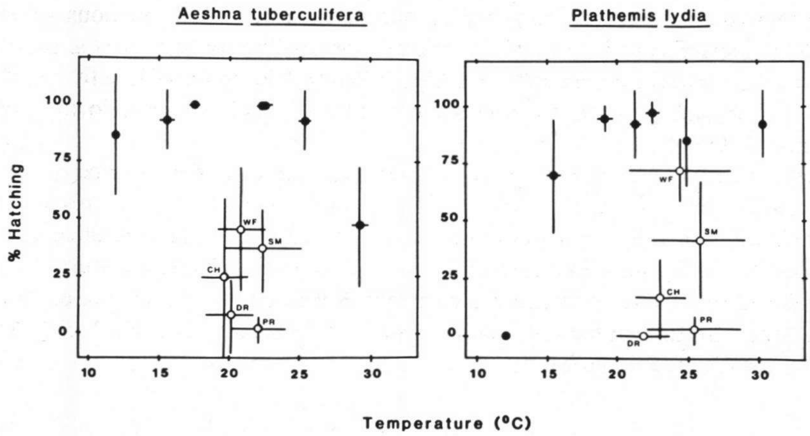

Fig. 2. Hatching success as a function of temperature for *Aeshna tuberculifera* and *Plathemis lydia*. Solid symbols represent the mean percent hatching (± 2 SE) from laboratory experiments in which temperature was controlled. Open circles represent values from the study ponds and are labelled accordingly. Pond temperatures are presented as the mean (± 1 SE) of the maximum and minimum temperatures recorded weekly during the period of embryogenesis.

at WF and SM ponds were close to the laboratory derived regression lines for embryogenic rate. Hatching success was also much lower in the field than in the laboratory (Fig. 2), with only the value for *P. lydia* at WF pond approaching hatching success values from the laboratory. In addition, there was no clear relationship between temperature and either hatching success or rate of embryogenesis in the field.

DISCUSSION

Although hyperbolic functions described the relationship between ln(Re) and temperature for both species in this study, the absolute rates of development for the two species cannot be compared. *A. tuberculifera* has an obligate embryonic diapause while *P. lydia* eggs develop directly following oviposition (HALVERSON, 1983). The *A. tuberculifera* eggs used in this study were collected in the spring roughly five months after deposition, and the embryos at this time already had well developed eyes which were visible through the egg chorion. Hence the rates reported for this species refer only to the final portion of embryonic development, while rates for *P. lydia* incorporate the entire embryonic period.

The effects of temperature on hatching success in the laboratory demonstrated contrasting temperature tolerances in these two species (Fig. 2). While egg hatching dropped at high temperatures in *A. tuberculifera*, success was lower at low temperatures in *P. lydia.* These differences are consistent with the geographical distributions and natural histories of the two species. *A. tuberculifera* is restricted to northern regions of North America (NEEDHAM & WESTFALL, 1955; WALKER, 1958) and embryonic development is completed in the early spring (Halverson, in review). *P. lydia,* on the other hand, occurs at more southern latitudes (NEEDHAM & WESTFALL, 1955) and passes through the egg stage in mid-summer.

Mean temperature was not an accurate predictor of embryogenic rate or hatching success in the field for either species (Figs 1 & 2). Values for both of these variables were lower in the field than predicted from laboratory results, indicating the importance of additional limiting factors in the study ponds. Furthermore, extensive variation in both embryogenic rate and hatching success occurred over a narrow range of mean field temperatures. This suggests that these additional limiting factors varied among ponds, thus obscuring the relationship between temperature and embryonic development in the field.

The failure of the laboratory derived temperature — development curves to provide a predictive model for the field is surprising in view of the success other workers have had in relating field and laboratory results (e.g. ELLIOTT & HUMPESCH, 1980; HUMPESCH & ELLIOTT, 1980; KANKAALA & WULFF, 1981; SMITH-GILL & BERVEN, 1979; BERVEN et al., 1979). The discrepancies between laboratory and field values in this study are of unknown origin and may involve any of a large number of physical, chemical, or biotic factors. One obvious difference between the laboratory and field environments was the extent of temperature fluctuations. In the laboratory, temperatures were held within a range of one to two centigrade degrees, while in the field, temperatures fluctuated extensively, both daily and seasonally. Evidence from the literature, however, suggests that temperature fluctuations per se have little effect on the developmental rates in most species (HOWE, 1967; SWEENEY & SCHNACK, 1977).

Another potential cause of the difference between field and laboratory results was the level of dissolved oxygen. Two observations indicate that this may have been the important factor in limiting embryogenesis at the study ponds. First, the highest hatching success for both species was achieved in the laboratory where oxygen levels were artificially elevated by aerating the water prior to the temperature experiments. Second, ponds in which hatching success and developmental rates were lowest, (i.e., DR, PR, and CH) also tended to have low levels of dissolved oxygen (Walton, pers. com.). Low oxygen levels could also explain the large discrepancies between field and laboratory values for *A. tuberculifera* relative to *P. lydia.* Oxygen used by the developing embryos is obtained by diffusion through the egg membrane. *A. tuberculifera* eggs are approximately four times the volume of *P. lydia* eggs resulting in a lower surface area to volume ratio. This presumably decreases the efficiency with which oxygen diffuses into the developing *A. tuberculifera* embryo, and could lead to a greater sensitivity to oxygen deprivation for this species.

CONCLUSIONS

Temperature had incontestable effects on both embryogenic rate and hatching success in the laboratory for both species. *A. tuberculifera* appeared to be more cold adapted than *P. lydia,* as would be expected from the geographical distributions and phenologies of the two species. However, no clear relationship between temperature and embryonic development was demonstrated in the field. This appears to be the result of an overriding influence of other limiting factors which varied among the study ponds. Some evidence suggests that the level of dissolved oxygen was more important than temperature in regulating embryonic development in the ponds in this study. Further experimental work on the effects of dissolved oxygen on embryogenesis under a variety of temperature conditions are needed to clarify the relative importance of oxygen level and temperature for embryonic development in odonates.

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